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Common HIV-1 Peptide Variants Mediate Differential Binding of KIR3DL1 to HLA-Bw4 Molecules[▽]

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Epidemiological studies have shown the protective effect of KIR3DL1/HLA-Bw4 genotypes in human immunodeficiency virus type 1 (HIV-1) infection; however, the functional correlates for the protective effect remain unknown. We investigated whether human leukocyte antigen (HLA)-Bw4-presented HIV-1 peptides could affect the interaction between the inhibitory natural killer (NK) cell receptor KIR3DL1 and its ligand HLA-Bw4. Distinct HIV-1 epitopes differentially modulated the binding of KIR3DL1 to HLA-Bw4. Furthermore, cytotoxic T lymphocyte (CTL) escape mutations within the immunodominant HLA-B57 (Bw4)-restricted Gag epitope TSTLQEQIGW abrogated KIR3DL1 binding to HLA-B57, suggesting that sensing of CTL escape variants by NK cells can contribute to the protective effect of the KIR3DL1/HLA-Bw4 compound genotype.

Epidemiological studies have demonstrated the strong influence of human leukocyte antigen (HLA) class I genes on the outcome of human immunodeficiency virus type 1 (HIV-1) infection (7). Control of HIV replication and slower disease progression are associated with HLA-B alleles expressing the serological Bw4 motif (HLA-Bw4) (10), which include HLA-B57 and HLA-B27. HLA-Bw4 molecules are ligands for receptors on T cells and natural killer (NK) cells and therefore can modulate both innate and adaptive immune responses. $CD8^+$ T cells recognize HLA class I-presented viral peptides through the T cell receptor (TCR) and are central for control of HIV-1 infection. It is now well established that HIV-1 can evade cytotoxic T lymphocyte (CTL)-mediated immune pressure through the selection of escape mutations within HLA class I-presented epitopes. In addition, HLA class I molecules also serve as ligands for the killer immunoglobulin-like receptors (KIRs), a family of receptors expressed on NK cells and T cells.

The role of KIR-HLA interactions in HIV-1 infection has received increasing attention over recent years. A significant association between slower HIV-1 disease progression and expression of the NK cell-activating receptor KIR3DS1 in conjunction with its putative ligand, HLA-Bw4 molecules that express isoleucine at position 80 (HLA-Bw4-80I), described the first association between NK cell receptors and HLA in modulating the clinical outcome of HIV infection (12). Although direct interaction between KIR3DS1 and HLA-Bw4-80I has not been demonstrated, NK cells expressing KIR3DS1 control HIV-1 replication in HLA-Bw4-80I-expressing target cells (1), and $KIR3DS1^+$ NK cells expand in acute HIV-1 infection preferentially in individuals that express HLA-Bw4-80I (2).

The combination of certain alleles of the inhibitory receptor KIR3DL1 and HLA-Bw4 has also been shown to be protective in HIV-1 infection (13). KIR3DL1 is highly polymorphic, and different allotypes result in different expression levels of KIR3DL1 on the cell surface of NK cells. A genetic study on a large cohort of HIV-1-infected individuals showed that individuals expressing KIR3DL1 allotypes that result in high levels of KIR3DL1 expression ($KIR3DL1^{hi}$), in conjunction with HLA-Bw4-80I, have significantly slower HIV-1 disease progression than individuals who express KIR3DL1 allotypes resulting in low levels of KIR3DL1 expression ($KIR3DL1^{lo}$), and that $KIR3DL1^{hi}$ strongly enhances protection conferred by HLA-Bw4-80I alleles alone (13). The functional correlates for the protective effect of the $KIR3DL1^{hi}$/HLA-Bw4-80I genotype in HIV-1 infection are not understood.

Several functional studies suggest that the interaction between KIR and cognate HLA class I can be modulated by the HLA-presented peptide (15) and that in particular amino acid residues 7 and 8 of the HLA-bound peptide can promote or prevent KIR interaction (15, 17). This was further supported by the resolution of the crystal structures of KIR2D receptors with HLA class I showing that KIR binds the α1 and α2 helix of major histocompatibility complex (MHC) class I and makes contact with the C-terminal end of the bound peptide (3). These structural and functional data suggest that changes in the HIV-1 epitopes presented by HLA class I during HIV-1 infection may affect KIR-HLA interactions and provide a potential mechanism for the protective effect of the $KIR3DL1^{hi}$/HLA-Bw4 genotype in HIV-1 infection.

We therefore investigated whether the common $KIR3DL1^{hi}$ allotype KIR3DL1*001 could discriminate between different HIV-1 epitopes presented by HLA-Bw4 molecules and show that different HIV-1 peptide variants can significantly modulate binding between KIR3DL1*001 and different HLA-Bw4/

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peptide tetramers. Furthermore, point mutations that are frequently selected by $CD8^+$ T cells within a dominant HLA-B57-restricted HIV-1 epitope in Gag abrogated binding of the inhibitory NK cell receptor KIR3DL1*001 to HLA-B*5701, suggesting that combined CTL- and NK cell-mediated immune pressure can contribute to the described protective effect of the $KIR3DL1^{hi}$/HLA-Bw4 compound genotype.

MATERIALS AND METHODS

Cell lines. Jurkat cells transfected with KIR3DL1*001-GFP (green fluorescent protein) were generated as previously described (14). Cells were cultured and maintained in growth medium (R10) containing RPMI medium 1640 (Gibco) supplemented with 10% heat-inactivated fetal calf serum (FCS), 1% penicillin-streptomycin (Sigma-Aldrich), and 2 mM L-glutamine (Sigma-Aldrich) at 37°C under 5% CO_2. Cells were cultured in 1 mg/ml G418 selection (Sigma-Aldrich) and maintained in logarithmic phase at neutral pH by diluting the cultures at a 1:10 dilution every 72 h.

Tetramer staining. KIR3DL1*001 Jurkat cells were stained with a panel of HLA class I tetramers refolded with HIV-1 peptides, including HLA-Bw4 tetramers (HLA-B*5701, HLA-B*2705, and HLA-A*2402) and HLA-Bw6 tetramers (HLA-B*08) as negative controls. Tetramer staining was verified using specific CTL clones (data not shown). Cells (2×10^5) were washed and resuspended in 100 μl wash buffer (phosphate-buffered saline [PBS] containing 1% FCS). Two microliters of phycoerythrin (PE)- or allophycocyanin (APC)-conjugated tetramers (Beckman Coulter) was added to KIR3DL1*001 Jurkat cells for 30 min at room temperature. Cells were washed and fixed in 1% paraformaldahyde until flow cytometric analysis was performed (FACSCanto; BD).

Statistical analysis. All experiments represent the means of results from five separate experiments. Unpaired *t* tests were used to determine statistical significance of differences. *P* values of <0.05 were considered significant.

RESULTS

Previous studies have shown that KIR3DL1 allotypes differ in their ability to bind HLA-Bw4 tetramers refolded with viral peptides (17). We investigated the ability of KIR3DL1*001, a KIR3DL1 allotype associated with slower disease progression in HIV-1 infection, to bind to different fluorescently labeled HLA-B tetramers refolded with HIV-1 peptides. KIR3DL1 binds HLA-B alleles with the serologically defined Bw4 epitope representing approximately 40% of HLA-B alleles. The remainder of HLA-B alleles carries the Bw6 motif (HLA-Bw6) and do not serve as ligands for KIR3DL1.

In accordance with the HLA-Bw4 allelic specificity of KIR3DL1, Jurkat cells transfected with KIR3DL1*001 bound HLA-Bw4 (HLA-B*5701, HLA-B*2705, and HLA-A*2402) but not HLA-Bw6 (HLA-B*0801) tetramers (Fig. 1a and b). Furthermore, the interaction between KIR3DL1*001 and the same HLA-Bw4 allele was influenced by different peptides (Fig. 1a and b). Strong binding of KIR3DL1*001 to HLA-B*5701 in the presence of some epitopes, including the $Gag_{147\text{-}158}$ ISPRTLNAW epitope and $Gag_{240\text{-}249}$ TSTLQE QIGW (TW10), was observed; however, HLA-B*5701 tetramers refolded with the $Gag_{162\text{-}172}$ KAFSPEVIPMF epitope bound weakly to KIR3DL1*001. The interaction between KIR3DL1 and HLA-B*2705 has been shown to be most sensitive to amino acid substitutions at positions 7 and 8 of a nonamer peptide (15), and charged residues at these positions are present in peptides that do not protect against lysis by $KIR3DL1^+$ NK cells. In line with this data, binding of KIR3DL1*001 to HLA-B*5701 was abrogated by the $RT_{244\text{-}252}$ IVLPEKDSW epitope ($P < 0.0001$; compared to binding of HLA-B*5701: TSTLQEQIGW to KIR3DL1*001), which carries a negatively charged aspartic acid residue at position 7. HLA-A*2402 carries the HLA-Bw4 motif and has been shown to bind KIR3DL1 (17). Binding of HLA-A*2402 tetramers to KIR3DL1*001 was also influenced by the presented peptide; HLA-A*2402:$Gag_{162\text{-}172}$ RDYVDRFFKTL bound strongly to KIR3DL1*001, while weaker binding of HLA-A*2402:$Gag_{28\text{-}36}$ KYRLKHIVW to KIR3DL1*001 was observed ($P < 0.0001$). These data demonstrate that the inhibitory NK cell receptor KIR3DL1 can discriminate between different HIV peptides presented by HLA-Bw4 molecules.

The immunodominant $Gag_{240\text{-}249}$TW10 epitope is one of the first HIV-1 epitopes targeted by $CD8^+$ T cells in HLA-B^*57^+ individuals during acute HIV-1 infection and one of the first epitopes to escape from $CD8^+$ T cell-mediated immune pressure (6, 11). Despite this escape from CTL-mediated immune pressure, HLA-B^*57^+ individuals, and in particular those that coencode $KIR3DL1^{hi}$, are highly enriched in cohorts of individuals with well-controlled HIV-1 infection (13). We investigated whether CTL escape mutations in the TW10 epitope at well-described positions of variation could affect the interaction between KIR3DL1*001 and HLA-B*5701 (Fig. 2a and b). KIR3DL1*001 bound strongly to the wild-type TSTLQE QIGW epitope; however, binding to HLA-B*5701 was significantly abrogated by the T242N mutation (TS**N**TLQEQIGW; bold letters indicate changes from the wild-type epitope) ($P < 0.0001$; compared to binding of HLA-B*5701: TSTLQEQIGW to KIR3DL1*001). KIR3DL1*001 binding to HLA-B*5701 was also significantly abrogated in the presence of two additional TW10 peptide variants that arise in HLA-$B57^+$ individuals: TS**N**LQEQI**A**W and TS**T**LQEQI**D**W ($P < 0.0001$; compared to binding of HLA-B*5701: TSTLQEQIGW to KIR3DL1*001). Thus, sequence mutations within the TW10 epitope that have been shown to be selected by $CD8^+$ T cells and that abrogate $CD8^+$ T cell recognition also reduce the binding of the inhibitory NK cell receptor KIR3DL1, potentially resulting in disinhibition of NK cells and rendering infected cells susceptible to NK cell lysis.

DISCUSSION

We have shown that binding of KIR3DL1*001, a KIR3DL1 allotype that results in high protein surface expression of KIR3DL1, to HLA-Bw4 molecules is modulated by the HLA-presented HIV-1 peptide. Binding of HLA-B*5701 to KIR3DL1*001 was promoted by $Gag_{147\text{-}158}$ ISPRTLNAW and $Gag_{240\text{-}249}$ TSTLQEQIGW peptides; however, weak binding and abrogation of binding was detected when HLA-B*5701 tetramers were refolded with $Gag_{162\text{-}172}$ KAFSPEVIPMF and $RT_{244\text{-}252}$ IVLPEKDSW, respectively. These data show that HIV-1 peptides can promote or prevent binding of HLA molecules to a specific KIR3DL1 molecule. HIV-1 peptides that abrogate inhibitory KIR-HLA interactions may promote recognition of an infected target cell *in vivo*, while HIV-1 peptides that induce strong inhibitory KIR-HLA interactions may allow escape from NK cell recognition and represent an immune evasion strategy for the virus, as recently suggested in the simian immunodeficiency virus (SIV) model (8). It has been shown that KIR^+ NK cells are activated more readily in response to peptide repertoire changes than to MHC class I downregulation (9). In this study, only a small peptide rep-

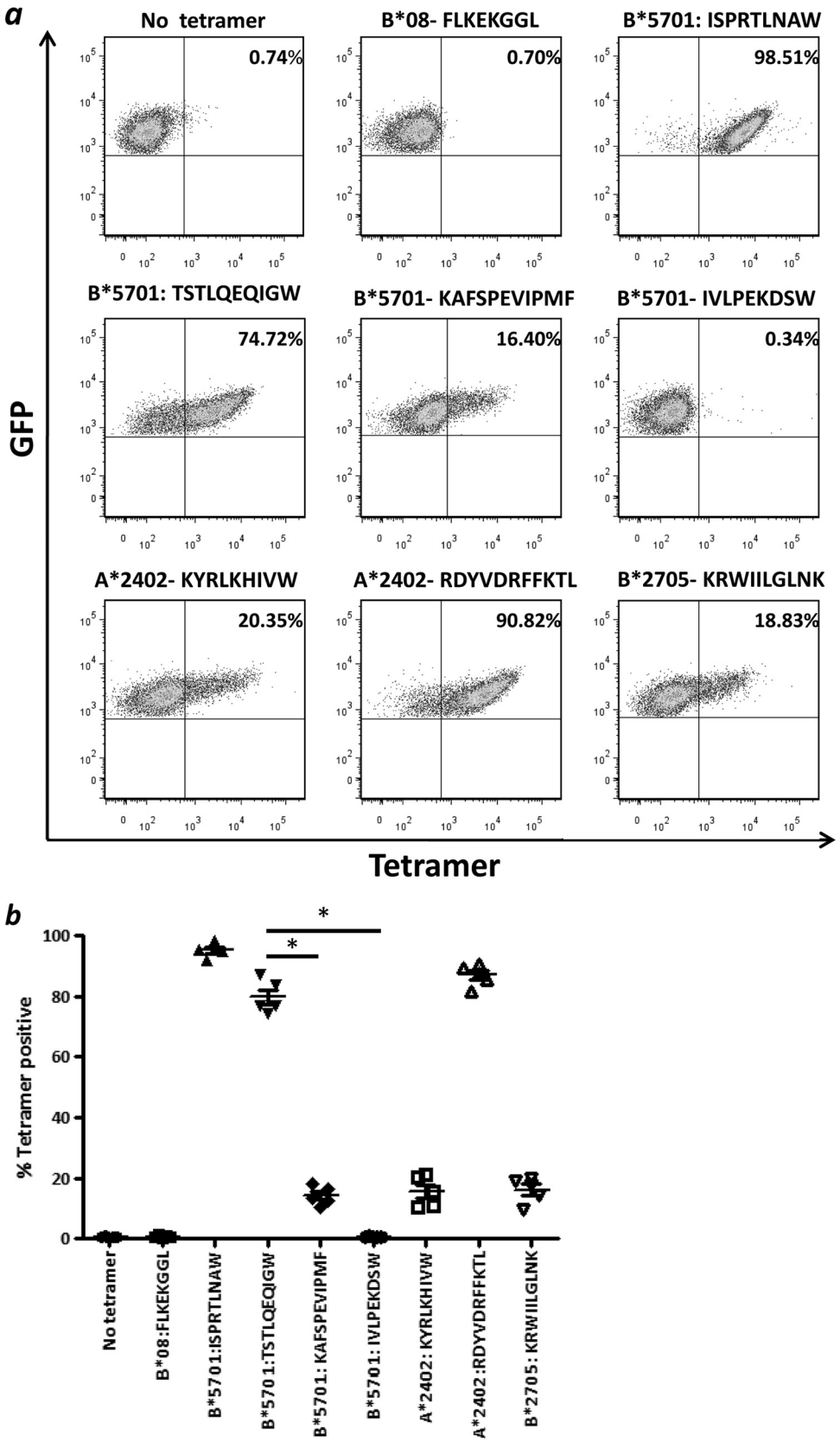

FIG. 1. Peptide-dependent binding of HLA class I tetramers to KIR3DL1*001. (a and b) Jurkat cells transfected with KIR3DL1*001-GFP were stained with HLA-Bw4 (HLA-B*5701, HLA-B*2705, and HLA-A*2402) or HLA-Bw6 (HLA-B*08) fluorescently labeled tetramers refolded with HIV-1 peptides. Tetramer binding was assessed by flow cytometry. (a) Representative flow-cytometric dot plots with percentages of tetramer-positive cells are shown. (b) Percentages of tetramer-positive KIR3DL1*001^{+} Jurkat cells are shown ±standard errors of the means (SEM) and are representative of results from five separate experiments. Statistically significant results are denoted by an asterisk (unpaired two-tailed *t* test).

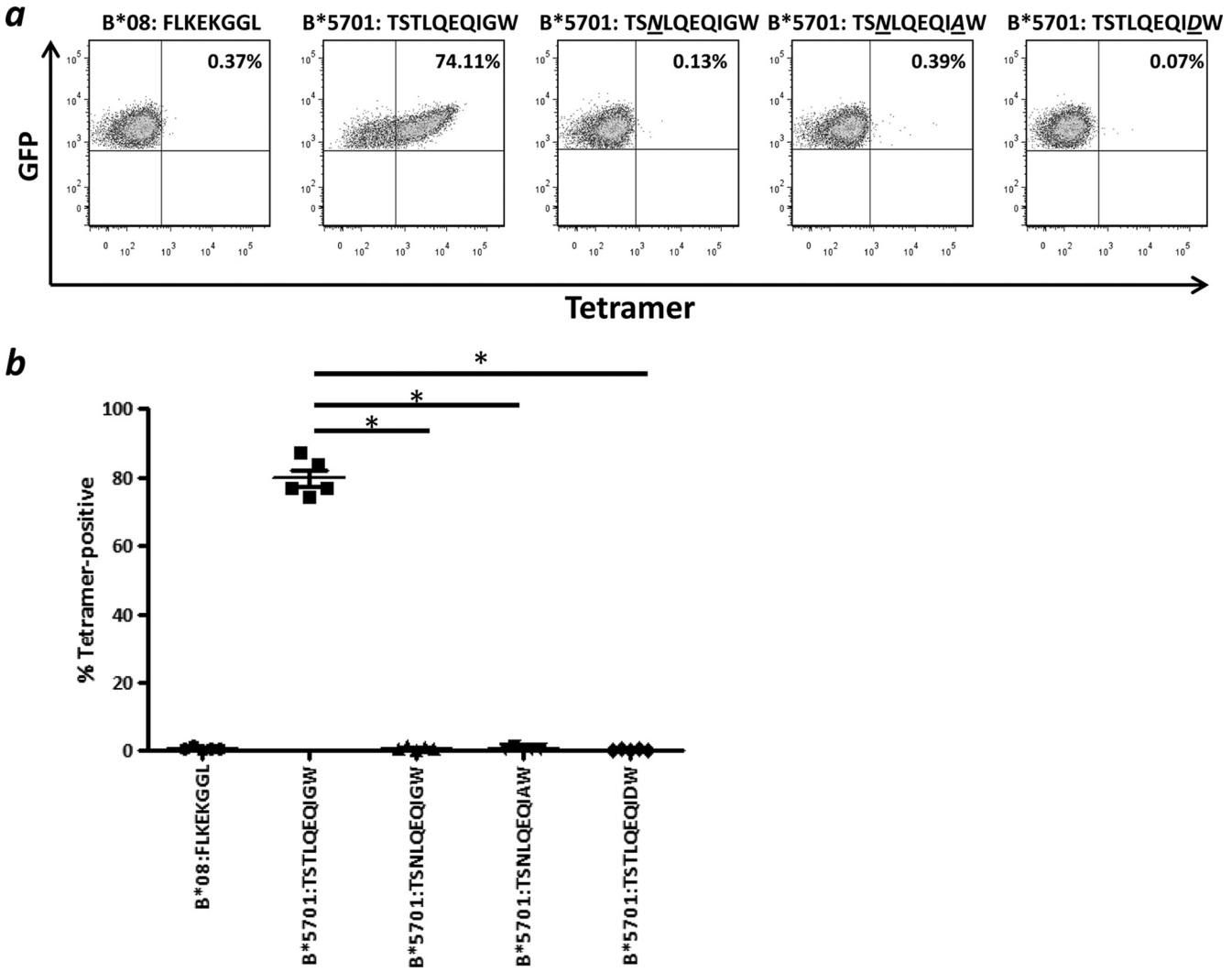

FIG. 2. Naturally occurring CTL escape mutations in TW10 epitope abrogate KIR3DL1*001 binding to HLA-B*5701. (a) Jurkat cells transfected with KIR3DL1*001-GFP were stained with HLA*B8: FLKEKGGL, HLA-B*5701 tetramers refolded with $Gag_{240-249}$ wild-type peptide TSTLQEQIGW, or mutant peptides (TSNTLQEQIGW, TSNTLQEQIAW, or TSTLQEQIDW). Tetramer binding was assessed by flow cytometry. (b) Percentages of tetramer-positive KIR3DL1*001$^+$ Jurkat cells are shown ±SEM and are representative of results from five separate experiments. Statistically significant results are denoted by an asterisk (unpaired two-tailed *t* test).

ertoire change toward peptides that are nonpermissive for KIR binding resulted in KIR$^+$ NK cell activation (9). Therefore, KIR3DL1^{hi} NK cells may respond strongly to small changes in peptide repertoire during HIV-1 infection if these peptides are nonpermissive for KIR binding, even in the presence of other virus- or host-derived peptides that do not interrupt KIR binding.

HIV-1 adaptation through amino acid point mutations to evade adaptive immune responses has been well described. The immunodominant HLA-B57-restricted $Gag_{240-249}$ TSTLQ EQIGW (TW10) epitope is targeted early in acute infection (16) but rapidly escapes in HLA-B57$^+$ individuals (6). The dominant sequence substitution is TS**N**LQEQIGW and is often associated with a further substitution at position 248, TS**N** LQEQI**A**W (11). Other mutations have been shown to arise in the TW10 epitope, including $Gag_{240-249}$ TSTLQEQI**D**W. We showed that while KIR3DL1*001 can bind to HLA-B*5701 in the presence of the wild-type TW10 peptide, this interaction is significantly reduced in the presence of all three TW10 mutants tested (TS**N**LQEQIGW, TS**N**LQEQI**A**W, and TSTLQE QI**D**W). Our observations that peptide changes within T cell epitopes can impact KIR3DL1 binding to HLA-B*5701 is in line with a recent study describing the consequences of sequence changes within HLA-B*5703-presented epitopes for KIR3DL1*002 binding (4), and further investigation into the functional implications of these modulations of KIR-HLA interactions is now required. Previous studies on KIR interaction with HLA have indicated that KIR binds the HLA-bound peptide at the C-terminal end (3, 15), and the change from a glutamine (G) to an alanine (A) or aspartic acid (D) in amino acid position 9 of the TW10 epitope may therefore prevent KIR interaction with HLA. The point mutation at position 242 of TW10 (TS**N**LQEQIGW) lies at the N-terminal end of the epitope, and how this peptide abrogates KIR recognition re-

quires further investigation. One explanation is that a point mutation at this N-terminal position may induce a change in the overall tertiary structure of the peptide affecting KIR binding at the C-terminal residues.

The TW10 epitope escapes rapidly in HLA-B*57^{+} individuals, though control of viremia in many of these individuals still persists. This has been suggested to be due to a reduction in viral fitness following escape (11), but *in vitro* data indicate that the reduction in viral fitness is minimal (5). In the context of the data presented here, it is possible that recognition of HLA-B*5701 by the inhibitory KIR3DL1^{hi} is lost in the presence of the TW10 escape mutations, resulting in activation of KIR3DL1^{+} NK cells and control of viremia. This model is consistent with the epidemiological data showing that individuals expressing HLA-B*57 and KIR3DL1^{hi} have slower disease progression than individuals expressing HLA-B*57 in the absence of KIR3DL1. In summary, these data provide novel insights into the potential mechanisms underlying the slower HIV-1 disease progression associated with the KIR3DL1^{hi}/HLA-B57 compound genotype.

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REFERENCES

1. **Alter, G., et al.** 2007. Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes. J. Exp. Med. **204:** 3027–3036.
2. **Alter, G., et al.** 2009. HLA class I subtype-dependent expansion of KIR3DS1+ and KIR3DL1+ NK cells during acute human immunodeficiency virus type 1 infection. J. Virol. **83:**6798–6805.
3. **Boyington, J. C., S. A. Motyka, P. Schuck, A. G. Brooks, and P. D. Sun.** 2000. Crystal structure of an NK cell immunoglobulin-like receptor in complex with its class I MHC ligand. Nature **405:**537–543.
4. **Brackenridge, S., et al.** 2011. An early HIV mutation within an HLA-B*57-restricted T cell epitope abrogates binding to the killer inhibitory receptor 3DL1. J. Virol. **85:**5415–5422.
5. **Brockman, M. A., et al.** 2007. Escape and compensation from early HLA-B57-mediated cytotoxic T-lymphocyte pressure on human immunodeficiency virus type 1 Gag alter capsid interactions with cyclophilin A. J. Virol. **81:** 12608–12618.
6. **Brumme, Z. L., et al.** 2008. Marked epitope- and allele-specific differences in rates of mutation in human immunodeficiency type 1 (HIV-1) Gag, Pol, and Nef cytotoxic T-lymphocyte epitopes in acute/early HIV-1 infection. J. Virol. **82:**9216–9227.
7. **Carrington, M., and S. J. O'Brien.** 2003. The influence of HLA genotype on AIDS. Annu. Rev. Med. **54:**535–551.
8. **Colantonio, A. D., et al.** 2011. KIR polymorphisms modulate peptide-dependent binding to an MHC class I ligand with a Bw6 motif. PLoS Pathog. **7:**e1001316.
9. **Fadda, L., et al.** 2010. Peptide antagonism as a mechanism for NK cell activation. Proc. Natl. Acad. Sci. U. S. A. **107:**10160–10165.
10. **Flores-Villanueva, P. O., et al.** 2001. Control of HIV-1 viremia and protection from AIDS are associated with HLA-Bw4 homozygosity. Proc. Natl. Acad. Sci. U. S. A. **98:**5140–5145.
11. **Leslie, A. J., et al.** 2004. HIV evolution: CTL escape mutation and reversion after transmission. Nat. Med. **10:**282–289.
12. **Martin, M. P., et al.** 2002. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. Nat. Genet. **31:**429–434.
13. **Martin, M. P., et al.** 2007. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. Nat. Genet. **39:**733–740.
14. **O'Connor, G. M., et al.** 2007. Functional polymorphism of the KIR3DL1/S1 receptor on human NK cells. J. Immunol. **178:**235–241.
15. **Peruzzi, M., K. C. Parker, E. O. Long, and M. S. Malnati.** 1996. Peptide sequence requirements for the recognition of HLA-B*2705 by specific natural killer cells. J. Immunol. **157:**3350–3356.
16. **Streeck, H., et al.** 2009. Human immunodeficiency virus type 1-specific CD8+ T-cell responses during primary infection are major determinants of the viral set point and loss of CD4+ T cells. J. Virol. **83:**7641–7648.
17. **Thananchai, H., et al.** 2007. Cutting edge: allele-specific and peptide-dependent interactions between KIR3DL1 and HLA-A and HLA-B. J. Immunol. **178:**33–37.